

(12) UK Patent Application (19) GB (11) 2 313 912 (13) A

(43) Date of A Publication 10.12.1997

(21) Application No 9611499.6

(22) Date of Filing 03.06.1996

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(51) INT CL⁶

C12Q 1/00, G01N 33/543

(52) UK CL (Edition O)

G1N NBEE N25B N25B3X N25DX

(56) Documents Cited

Elecroanalysis, Vol. 6, Is. 11-12, 1994, pages 934-944,
 Kutner W et. al.

(58) Field of Search

UK CL (Edition O) G1N NBEE NBPE

INT CL⁶ C12Q 1/00, G01N 33/543

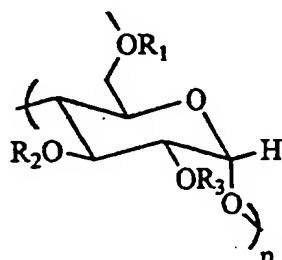
ONLINE: BIOSIS, CA SEARCH, CAB ABSTRACTS, WPI

(54) Thin film biosensor

(57) A biosensor comprises an electrode coated with a thin film made up of, a lipophilic cyclodextrin derivative of figure 1 wherein n = 6, 7 or 8 and R1, R2 and R3 which may be the same or different, are alkyl, aralkyl, aryl or acyl groups optionally substituted in any position, a charge shuttle or electron mediator such as a ferrocene derivative, a redox protein, tetrathiafulvalene, any of its derivatives or charge-transfer complexes, one or more enzymes or antibodies selected to bind selectively or specifically to a given molecule or ion, a plasticised polymeric matrix and a lipophilic anion as its salt.

The biosensor can be used for the detection and/or monitoring of a molecule or ion which can bind to the enzyme/antibody and/or to the cyclodextrin derivative, or for the monitoring of compounds or ions which inhibit the activity of the enzyme.

Fig 1

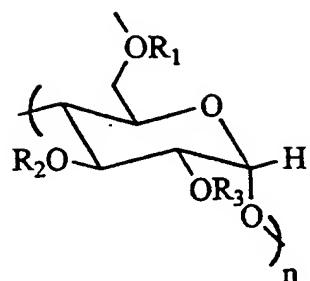


R₁, R₂, R₃ : Alkyl groups

n = 6,7 or 8 corresponding to α , β or γ cyclodextrins

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Fig 1



R_1, R_2, R_3 : Alkyl groups
 $n = 6, 7$ or 8 corresponding to α , β or γ cyclodextrins

Fig 2

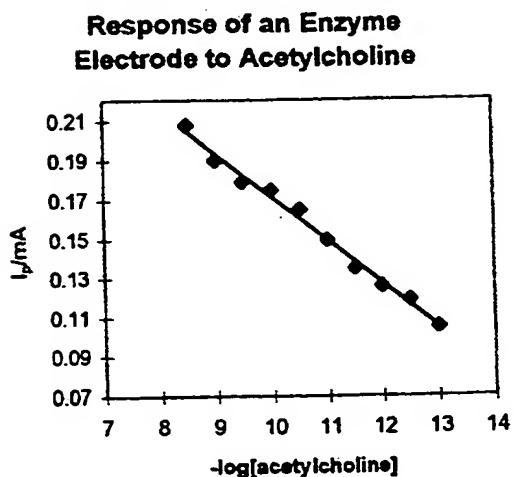


Fig3a

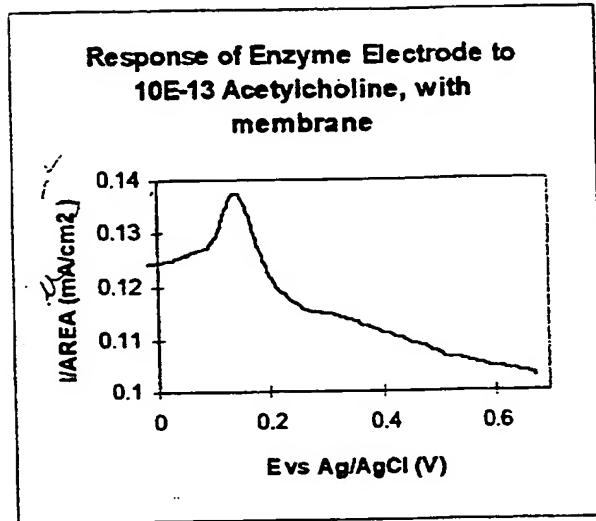


Fig 3b

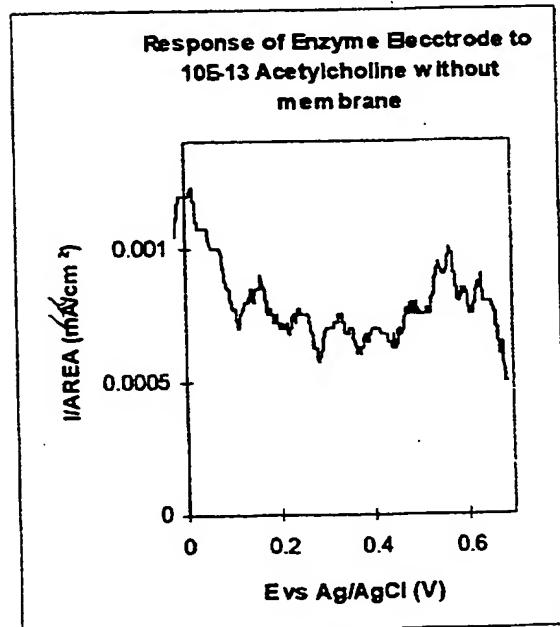


Fig 4a

ACH: acetylcholine
 SHIAA: 5 hydroxy indole 3 acetic acid
 DOP: dopamine
 ACM: acetaminophen
 AA: ascorbic acid

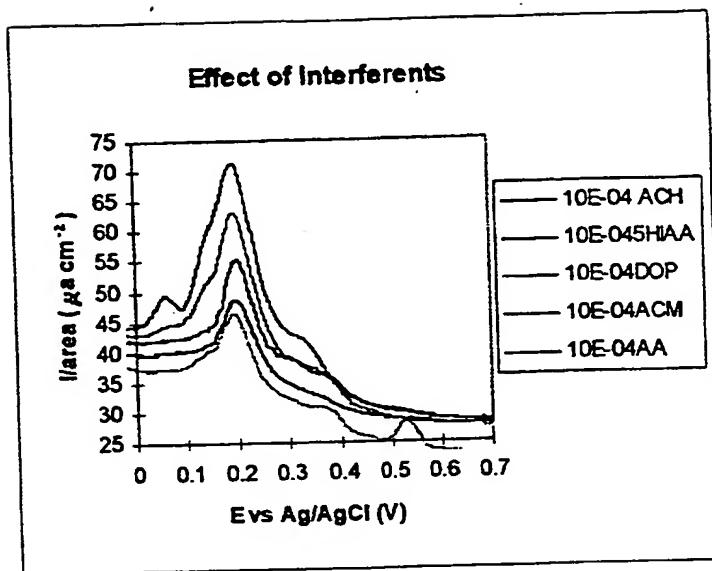
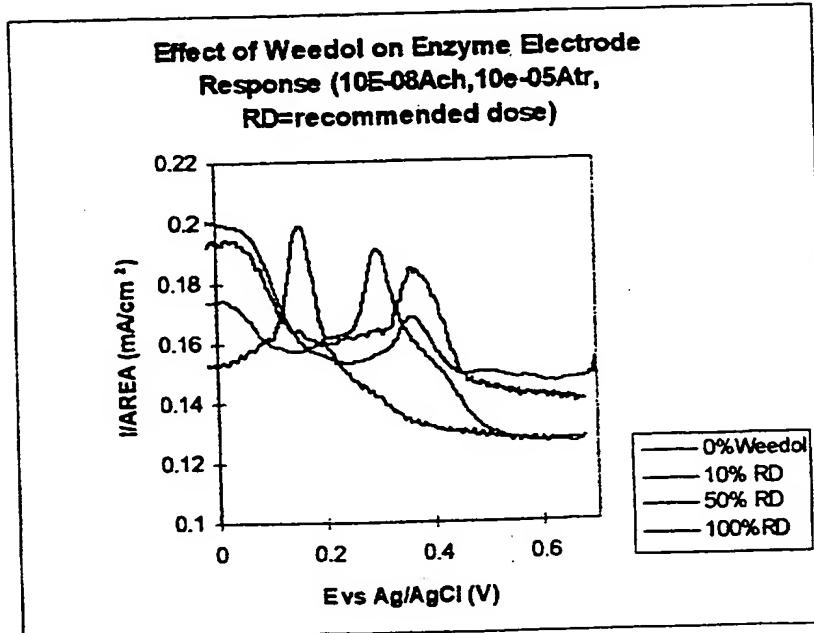


Fig 4b



THIN FILM TECHNOLOGY FOR THE FABRICATION OF SENSITIVE, ROBUST BIOSENSORS

Field of Invention:

This invention relates to sensors and to the formulation of a thin film composite comprising a lipophilic cyclodextrin, a suitable polymer matrix, a plasticiser, a charge shuttle and a large anionic salt which deposited over an enzyme or immunosensor allows sub picomolar levels of detection. At the same time the film shields the underlying proteins from denaturation for at least five months when stored dry.

Background:

Biosensor technology is being developed as a means of providing sensitive, selective, robust devices for the detection of analytes in pharmaceutical, clinical, forensic and environmental laboratories. Electrochemical sensors reported to date typically have micromolar to nanomolar levels of detection. Detection at lower levels require preconcentration techniques such as microdialysis or HPLC separations [see for example W.J. Albery et al., Phil. Trans. R. Soc. Lond. A; 1990; 333:49-61]. These devices have the additional disadvantage of losing their activity following prolonged storage.

Lipophilic cyclodextrins have been found to be suitable ionophores for the complexation and detection of a range of alkyl and aryl ammonium analytes [see, for example R. Kataky et al. Scan. J. Clin. Lab. Invest., 1995; 55:409-419, P.S. Bates et al., J. Chem. Soc. Perkin Trans. 2, 1994; 669-675]. The parent cyclodextrin molecules, with hydroxy groups in the 2, 3 and 6 positions, are made up of 6 (α), 7 (β) or 8 (γ) glucose units and form well-defined inclusion complexes with a variety of aromatic and aliphatic molecules and ions. The size of the cavity is important and the volume it defines should match that of a potential substrate for inclusion. Some degree of complexation selectivity may then be obtained by taking advantage of this size complementarity.

A problem in using such cyclodextrins or their derivatives as ionophores for the selective detection of charged analytes is that they generally possess only modest selectivity in binding a cationic guest molecule, even though they may show excellent selectivity over charge dense cations such as sodium, potassium, magnesium and calcium. We now report a means of overcoming this problem by introducing a suitable lipophilic cyclodextrin and a charge relay into a sensor formulation that also includes an enzyme or antibody. The resultant coupled biosensor exhibits excellent sensor characteristics (substrate specificity, low limits of detection, stability to storage) that may allow its usage in a variety of pharmaceutical, clinical, environmental or forensic assays.

Thus, according to one aspect of the invention, we provide an electrode assembly which comprises a working electrode such as a screen-printed substrate with a carbon working electrode over which is deposited a thin film of an electron mediator (such as ferrocene, or any substituted ferrocene derivative, tetrathiafulvalene, its derivatives or any of its charge transfer complexes, and/or a redox-protein), a suitable combination of enzymes or antibodies selected to bind a given molecule or ion, such as acetyl choline esterase, choline oxidase, dopamine decarboxylase, and covered with a thin film composite comprising a polymer matrix, such as polyvinylchloride or preferably polyurethane, a plasticiser such as o-nitrophenyl octyl ether, bis-(butylpentyl)-adipate or di-octylsebacate, a large anionic salt such as potassium tetrakis(p-chlorophenylborate) or sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate and a lipophilic cyclodextrin, (Figure 1). The lipophilic cyclodextrin which may contain 6, 7 or 8 glucose units ($n = 6, 7, 8$) is substituted at the positions shown wherein R_1 , R_2 and R_3 which may be the same or different are alkyl, aralkyl, aryl or acyl groups, optionally substituted in any position; preferably R_1 , R_2 and R_3 are linear or branched alkyl groups and preferred combinations include R_1 the same as R_2 , with R_3 being hydrogen or the same or a different alkyl group and in particular $R_1 = R_2 =$

$C_{12}H_{25}$ when $R_3 = H$, $R_1 = R_2 = R_3 = C_8H_{17}$ and $R_1 = R_2 = R_3 = \text{ethyl}$ when n is seven (a β cyclodextrin derivative). This electrode assembly allows sub-picomolar levels of analyte to be detected amperometrically with high selectivity, (Figure 2). The resultant devices exhibit a stable response and may be stored in air for at least several months without losing their activity.

In another aspect of the invention, related electrode assemblies formulated without the addition of a lipophilic cyclodextrin derivative are relatively insensitive in detecting the given analyte: Thus for example electrode assemblies prepared with and without the addition of a lipophilic cyclodextrin derivative show very different behaviour: the assembly that includes the lipophilic cyclodextrin derivative in its formulation exhibits a measurable response to low levels of analyte (e.g. acetyl choline in Figure 3a, detected at a concentration of $10^{-13} \text{ mol dm}^{-3}$) whereas assemblies lacking the cyclodextrin derivative and the encapsulating film show no measurable response to the acetyl choline analyte at the same concentration, (Figure 3b).

In yet another aspect of the invention, we provide the use of a sensor for the selective detection of molecules or ions that may bind to the active site of one of the enzymes used in the formulation of the sensor. Well defined inhibitors may compete with a sample of the typical enzyme substrate and hence diminish enzymic activity, allowing their presence to be detected and/or monitored selectively. Thus in a sensor that includes acetyl-choline esterase, the detection of acetyl choline at varying concentrations will be affected by the presence of species known to bind to the enzyme active site or inhibit the enzyme's activity, such as various herbicides, in particular paraquat or diquat, well-defined antagonists of the enzyme receptor such as neostigmine, and nerve gases such as Sarin or Dyflos.

Example 1

Formulation of a sensor for the sensitive detection of acetyl choline.

A biosensor was formulated based on the well established horseradish peroxidase/choline oxidase/acetylcholine esterase system which allows micromolar levels of detection of acetylcholine via the amperometric detection of hydrogen peroxide relayed by a ferrocene derivative. A thin film of an electron rich ferrocene derivative such as 0.1 mol dm^{-3} 1,1'-bis methoxymethyl ferrocene in acetonitrile was deposited on the carbon working electrode of a screen printed substrate and allowed to dry. A combination of 5000U horseradish peroxidase, 100U choline oxidase and 200U acetylcholine esterase in 0.05 mol dm^{-1} , pH 7, phosphate buffer formed the next layer. Over this was deposited a thin film comprising 40% (w/w) Tecoflex SG80, 59.5% (w/w) plasticiser (bisbutylpentyl adipate or ortho-nitro phenyl octyl ether), 4.8% (w/w) ionophore (triethyl β cyclodextrin or 2,6 didodecyl β cyclodextrin) and 0.5% (w/w) sodium tetrakis [3,5-bis(trifluoromethyl)phenyl] borate. This assembly formed a dip type sensor ready for use, that could be stored in air for periods of several months prior to use.

The electrode was allowed to condition in phosphate buffer for two hours prior to use to allow the thin film to be wetted and the enzymes to swell. Sub-picomolar levels of acetyl choline were detected with no detrimental interference from compounds of the type ascorbic acid, dopamine, 5-hydroxy indole 3-acetic acid (5 HIAA) acetaminophen and atropine [Figure 4a]. The electrode was also very convenient to use as a dip type sensor for detecting pesticides such as paraquat and diquat which are acetylcholine esterase inhibitors [Figure 4b]. Thus addition of 10% of the recommended dose of the commercial herbicide Weedol (equivalent to a concentration of 60 mg per litre), inhibited observation of the ferrocene/ferrocenium oxidation wave at +170 mV (vs. Ag/AgCl).

Claims

1. A biosensor comprising an electrode coated with a thin film made up of a lipophilic cyclodextrin derivative of formula 1, wherein n = 6, 7 or 8 and R₁, R₂ and R₃ which may be the same or different, are alkyl, aralkyl, aryl or acyl groups optionally substituted in any position, a charge shuttle or electron mediator such as a ferrocene derivative, a redox protein, tetrathiafulvalene, any of its derivatives or charge-transfer complexes, one or more enzymes or antibodies selected to bind selectively or specifically to a given molecule or ion, a plasticised polymeric matrix and a lipophilic anion as its salt, for use as an analytical method for the detection and/or monitoring of a molecule or ion which may bind to the enzyme and/or to the cyclodextrin derivative.
2. A sensor, formulated according to claim 1, which involves the enzymes, acetyl choline esterase, choline oxidase and horseradish peroxidase, a lipophilic cyclodextrin wherein n = 6 or 7, 8, R₁ = R₂ = C₁₂H₂₅, R₃ = H or R₁ = R₂ = R₃ = Et and a ferrocene derivative for use in the selective detection of acetyl choline.
3. A sensor for use in the selective monitoring or detection of compounds or ions that are known to inhibit the activity of the enzyme, claimed in Claim 1.

Amendments to the claims have been filed as follows

1. A biosensor comprising an electrode coated with a thin film made up of:
 - a lipophilic cyclodextrin derivative to the formula of Figure 1, wherein n = 6, 7 or 8 and R₁, R₂ and R₃ which may be the same or different, are alkyl, aralkyl, aryl or acyl groups optionally substituted in any position;
 - a charge shuttle or electron mediator such as a ferrocene derivative, a redox protein, tetrathiafulvalene, any of its derivatives or charge-transfer complexes;
 - one or more enzymes or antibodies selected to bind selectively or specifically to a given molecule or ion;
 - a plasticised polymeric matrix and a lipophilic anion as its salt.
2. A sensor, formulated according to claim 1, which involves the enzymes, acetyl choline esterase, choline oxidase and horseradish peroxidase, a lipophilic cyclodextrin wherein n = 6 or 7, 8, R₁ = R₂ = C₁₂H₂₅, R₃ = H or R₁ = R₂ = R₃ = Et and a ferrocene derivative.
3. The use of the biosensor, according to claim 1, in an analytical method for the monitoring and/or detection of a molecule or ion which binds to the enzyme and/or to the cyclodextrin derivative in the sensor.
4. The use of the biosensor, according to claim 1, where the biosensor contains an enzyme, in the selective monitoring and/or detection of compounds or ions which are known to inhibit the activity of the enzyme in the biosensor.
5. The use of the biosensor, according to claim 2, in the selective detection of acetyl choline.



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Application No: GB 9611499.6
Claims searched: 1-3

Examiner: David Mobbs
Date of search: 13 August 1997

Patents Act 1977
Search Report under Section 17

Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK CI (Ed.O): G1N NBEE, NBPE.

Int Cl (Ed.6): C12Q 1/00; G01N 33/543.

Other: ONLINE: BIOSIS, CA SEARCH, CAB ABSTRACTS, WPI.

Documents considered to be relevant:

Category	Identity of document and relevant passage	Relevant to claims
A	Electroanalysis, vol. 6, Is 11-12, 1994, pages 934-944, Kutner W et al., Condensation alpha-cyclodextrin polymer membrane with covalently immobilised glucose oxidase and molecularly included mediator for amperometric glucose biosensor.	